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LARVAL RELEASE IN RESPONSE TO A LIGHT SIGNAL BY THE INTERTIDAL SPONGE *HALICHONDRIA PANICEA*

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ABSTRACT

The intertidal sponge, *Halichondria panicea,* regularly begins releasing larvae shortly after dawn, and ejects most of them during morning hours under a natural light-dark (LD) cycle. Its diurnal periodicity was confirmed under an artificial LD 12:12h cycle. In search of a trigger that stimulates the sponge colonies to release larvae, the colonies were subjected to experimentally modified LD cycles. Under continuous darkness, only a single release peak was observed about fifteen hours after the beginning of darkness. Further, the colonies invariably released larvae about fifteen hours after the change from light to dark on the preceding day under all illumination regimes examined. The timing of their larval release was independent of both the tidal cycle and the daily cycle of seawater temperature. These results indicate that the trigger is a light signal: the onset of darkness (not onset of light) of the preceding day under natural illumination. Subsequent to this stimulus, *H. panicea* needs a period of fifteen hours before release actually occurs. This light-controlled larval release probably has ecological significance for habitat selection by intertidal sponges.

INTRODUCTION

Most intertidal sponges are viviparous (Hyman, 1940; Bergquist *et al.,* 1970). In these sponges, fertilized eggs develop into swimming larvae within the mesohyle, and then leave the parent sponge through the excurrent canals and oscula (Levi, 1956; Fell, 1969; Fell and Jacob, 1979). The ubiquity of viviparity among intertidal sponges suggests some ecological significance for their habitat selection. However, little is known about the larval release mechanism.

About thirty years ago, Levi (1951, 1956) showed that larval release occurs shortly after sunrise in several sponge species. Another species, however, released larvae throughout the day following their collection (Levi, 1956). According to incidental observations, several sponges extruded larvae for several hours after collection (Wilson, 1894; Ali, 1956; Fell, 1967, 1969; Fell and Jacob, 1979). In such instances, the release may be induced artificially by shock or confinement.

Halichondria panicea released the majority of its larvae during morning hours under natural illumination in our laboratory. Using experimental light regimes, the study described in the present paper demonstrates clearly the role of light in inducing larval release in *H. panicea.* The results facilitate a discussion of habitat selection by sponges from an ecological point of view.

MATERIALS AND METHODS

All *Halichondria panicea* colonies were collected from the shallow waters near the Asamushi Marine Biological Laboratory in Japan (40° 67′N: 140° 52′E). The greenish

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colonies, which are several centimeters thick, usually encrust rocks which are both exposed to the sun and washed by waves. Because these colonies attach broadly and firmly to the rocks, a sharp knife-blade was used carefully to free the colonies from the substrate, so as to minimize any damage. The freed sponges were transferred under water to individual water-tight containers and immediately brought to the laboratory. There they were placed quickly in running seawater. The sponge colonies treated in this manner appeared healthy and showed little degeneration during the laboratory investigations.

Early in the morning following collection, the sponge colonies were placed individually in still seawater. After one hour, those releasing a large number of larvae were selected for study. Only a small proportion of the collected colonies (about 20%) released many larvae in the laboratory.

For the study of larval release under various illumination schedules, a photographic developing tank was used as a light-shielding container for each sponge colony. The colony was fastened inside the tank and continuously supplied with fresh, clean running seawater while it was illuminated or completely shielded from light. The colonies were illuminated by a fluorescent light (40W $\times$ 2) mounted on the ceiling of the laboratory. The released larvae were washed by the outflow into a larva collector. The larva collector is a plastic vessel with a piece of nylon mesh (NXX 13, 94 μm) applied to a pore (about 3 $\times$ 5 cm) on one side of the vessel. The collected larvae were counted regularly.

Results

Halichondria panicea colonies released larvae in August and September. Similarly, several other sponges release larvae in the later period of their reproductive season (Simpson, 1968; Chen, 1976; Fell and Jacob, 1979; Ayling, 1980). The larvae of this species are pale yellow and their size is about 400 $\times$ 250 μm. They are thickly covered with cilia except for a posterior pole which is bare. The bare pole is encircled by a band of long cilia. These parenchymula larvae swim rapidly with constant rotation as soon as ejected from the osculum.

In the laboratory early the next morning after collection, I found several *H. panicea* colonies releasing many larvae. Thus larval release was first studied under natural illumination. Usually, larval release began within one hour after dawn (about 5:30) and the majority of larvae were released before noon. A small number of larvae were released in the afternoon and almost none were released after dusk (about 18:30) until the next morning. This concentrated morning release was repeated during successive days.

This diurnal periodicity was confirmed under the artificial light-dark cycle (LD 12:12h, light period 6:00 to 18:00). A typical example for 24 hours is shown in Figure 1, since all the colonies examined released larvae in an essentially similar pattern. Under this LD cycle, the sponge colony began releasing larvae shortly after the beginning of the light period; release regularly peaked after a few hours (about 9:00). Most larvae were released during the first half of the light period (6:00–12:00) with fewer released in the latter half (12:00–18:00). Almost none were released in the dark period (18:00–6:00). This periodical release continued for more than a week (Fig. 2).

During these experiments, the seawater temperature changed diurnally (Fig. 1). Usually the temperature was high in the afternoon, low at night, and its difference within a day was about two degrees.

To investigate the mechanism that triggers the diurnal periodicity of larval release, the sponge colony was subjected to experimentally modified LD schedules. Figure 3

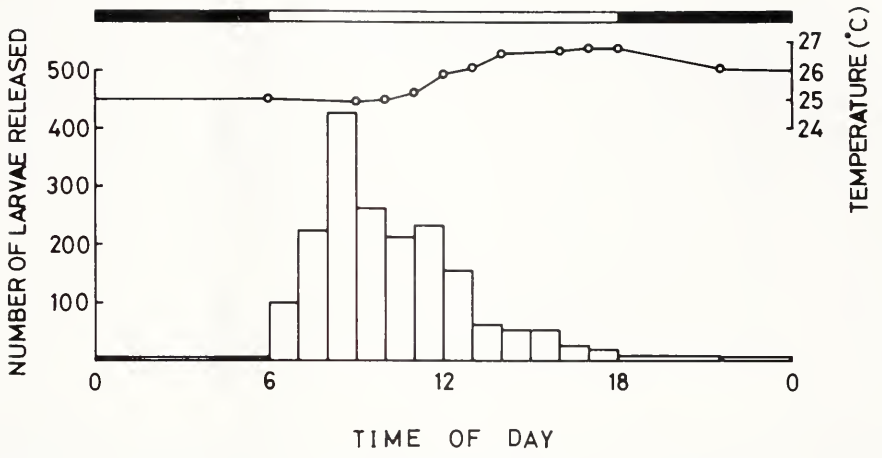

FIGURE 1. Typical pattern of larval release by *Halichondria panicea* during 24 hours under a LD 12:12h cycle. Included is the change in seawater temperature on that day. At the top, the illumination schedule is shown. The blackened bar indicates the dark period and the white portion, the light period.

shows the effect of continuous darkness on larval release. Here a sponge colony that had been under the LD 12:12h cycle was subsequently kept in the dark for 54 hours. Under continuous darkness, only a single peak of release occurred and no second peak was observed as long as darkness continued. Characteristically, the release peaked about 15 hours after the beginning of continuous darkness; this time corresponds exactly to when a peak would occur if the LD 12:12h cycle had been continued. This result shows that, first, even in the dark the sponge colony is initially able to release larvae and light is not necessary. Second, the release of larvae is not controlled by the circadian rhythm. Third, the stimulus that induces release is not the dawning light of that morning. Thus it is reasonable to conclude that the illumination on the preceding day determines the time of release.

Figure 4 shows larval release when the timing of the LD cycle on the preceding

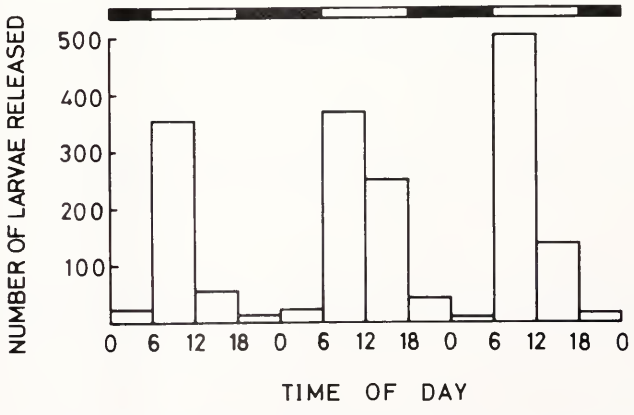

FIGURE 2. Diurnal periodicity of larval release by *Halichondria panicea* during three successive days under the LD 12:12h cycle. A release peak appeared regularly each day at about 9:00.

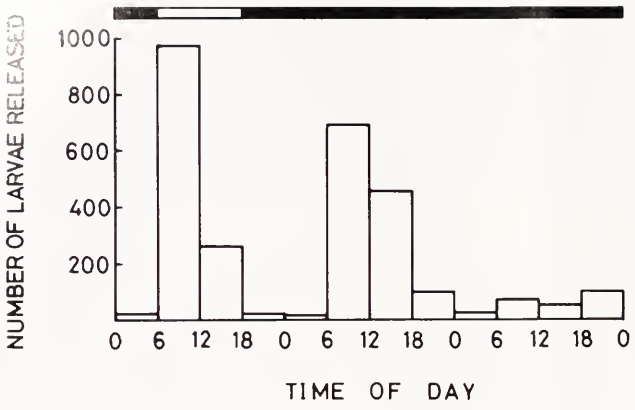

FIGURE 3. Larval release under continuous darkness. On the first day, the colony was illuminated (6:00–18:00) as in the LD 12:12h cycle then subsequently darkened for 54 hours. On the second day although it was dark, it released larvae at the same time as if still under the LD 12:12h cycle. On the third day, however, no concentrated release was observed.

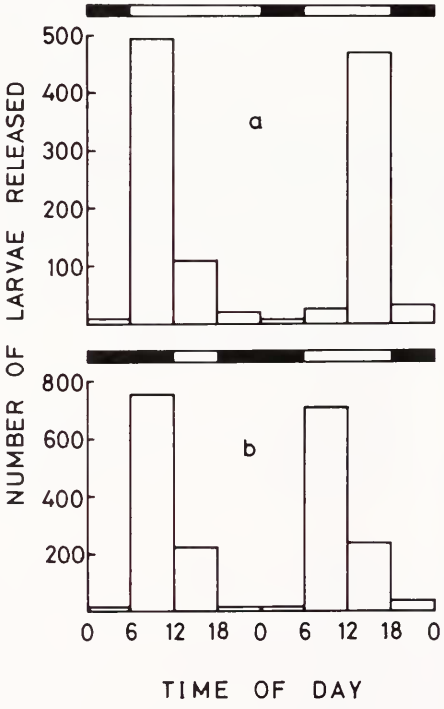

FIGURE 4. Larval release under modified light-dark cycles. (a) Delay in the onset of the dark period for six hours resulted in a delay of release for six hours on the next day. (b) Six-hour delay in the onset of the light period, however, did not bring about any delay in the next day's release.

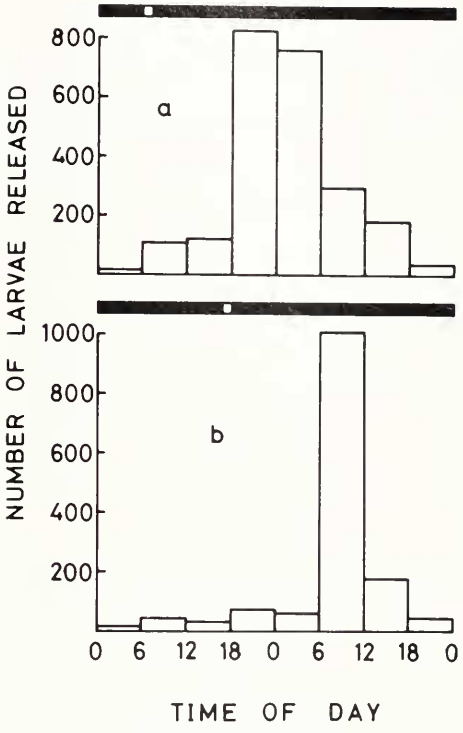

FIGURE 5. Larval release induced by illumination for one hour. (a) One hour of illumination in the morning (6:00–7:00) brought about a release peak at midnight. (b) One hour of illumination in the evening (17:00–18:00) brought about a release peak in the next morning. In both cases, the peaks appeared about fifteen hours after illumination.

day was modified. Figure 4a shows that a six-hour delay in the onset of the dark period on the preceding day resulted in delay of larval release for six hours. On the other hand, a six-hour delay in the onset of the light period had no effect (Fig. 4b). Thus, it is highly probable that the timing of larval release is determined by the change from light to dark (L to D) in the LD cycle on the preceding day.

Results presented in Figure 5 verify the above supposition. Preliminary experiments showed that illumination for one hour is sufficient to induce dark-adapted sponges to release larvae. If the trigger is really the L to D change in the preceding LD cycle, larvae should be released about 15 hours (18:00–9:00 in LD 12:12h cycle) after the one hour illumination. Figure 5 clearly indicates that this is the case because the peaks appeared after about 15 hours whether the sponge colonies were illuminated at dawn (6:00–7:00) or at dusk (17:00–18:00).

Table I summarizes the results graphed in Figures 2 to 5. Under the various LD regimes, the duration between the time of L to D change in a preceding LD cycle and the peak of larval release is always approximately fifteen hours. On the other hand, D to L changes of that morning or on the preceding day had no constant temporal relationship to the releases. These results indicate that the darkening at dusk is the true stimulus under natural illumination and that a delay of fifteen hours is necessary before larval release can occur. Furthermore, the length of the light periods (1–18 h) is apparently without effect under these experimental conditions.

TABLE I

Temporal relationship between light signals and larval release in Halichondria panicea *under various LD regimes*

D to L on that day*	L to D on the preceding day*	D to L on the preceding day*	Reference
3	15	27	Fig. 2
—	15	27	Fig. 3**
9	15	33	Fig. 4a**
3	15	21	Fig. 4b**
—	14	15	Fig. 5a
—	15	16	Fig. 5b

* Approximate durations (hours) between the time of light onset (D to L) or dark onset (L to D) and the peak of larval release.

** Peak of release on the second day.

DISCUSSION

Under natural illumination in the laboratory, *Halichondria panicea* colonies release the parenchymula larvae with diurnal periodicity: they release the majority of larvae during morning hours. *Haliclona permolis* also releases larvae in the morning (Amano, unpub. data). Similarly, Levi (1951, 1956) reported the release of larvae a short time after sunrise in *Oscarella lobularis, Hymeniacidon sanguinea,* and *Halisarca metschnikovi.* These five species represent most viviparous orders of the class Demospongiae. So although additional studies obviously are necessary, the concentrated release of larvae in the morning may be a common phenomenon among the Demospongiae.

H. panicea colonies were subjected to artificial light regimes in a search of an afterdawn larval release trigger. Results from these studies clearly indicate that the larval release of *H. panicea* is triggered by a light signal: the onset of darkness on the preceding day under natural illumination. Unfortunately, it is as yet unknown whether this light-controlled release is common among other sponges. Many colonial ascidians also release their tadpole larvae during morning hours under natural illumination. The release is also controlled by a light signal; however, it is not the onset of dark but the onset of light that triggers the release (Watanabe and Lambert, 1973). Why do these triggers differ while bringing about similar release patterns? The two patterns may have been acquired independently as a result of convergence in the remotely related phyla.

No environmental elements other than light exhibited any detectable influence upon the timing of larval release by *H. panicea.* It is independent of the daily cycle of seawater temperature and of the tidal cycle as well. Furthermore, it is unlikely that timing is controlled by the circadian rhythm since only a single peak of release was observed during continuous darkness.

Intertidal sponges may remain healthy for some time in the laboratory if maintained in suitable conditions (Fell, 1967). Sponge colonies must be collected carefully so as to minimize deleterious effects and constantly supplied with fresh, clean running seawater during the experiments. Colonies treated in this manner always reacted to the light signals for more than two weeks. Therefore, although results of field studies are not available for comparison, it is reasonable to consider that the larval releases observed in this study are not caused by uncontrollable artifacts (Wilson, 1894; Ali,

1956; Fell, 1967, 1969; Fell and Jacob, 1979) but reflect the release in their natural habitat.

Why do the sponges release larvae during the morning hours? As noted before, many colonial ascidians release larvae primarily in the morning (Watanabe and Lambert, 1973). Symbionts of algal-ascidians, however, release at midday (Olson, 1983). The behavior and settlement of these tadpole larvae are closely attuned to light conditions, with the larvae settling in shaded habitats (Millar, 1971). In the corals, planula larvae are positively phototactic upon release but later reverse this and become attracted to dark surfaces (Harrigan, 1972; Lewis, 1974). Because their free-swimming periods are relatively short (several hours in most cases), if released in the morning, the larvae should have a maximum period for seeking a suitable habitat during the day of release. The larvae of *H. panicea* swim rapidly for two days after release and are positively phototactic during this swimming phase. Several hours before settlement, however, their behavior changes; they creep around the substratum and are indifferent to light (Amano, unpub. obs.). On the other hand, adult sponges of this species usually inhabit the surfaces of rocks relatively exposed to the sun. Although larvae of many sponges can respond to light and gravity they do not have consistent behavior patterns (Warburton, 1966; Bergquist *et al.,* 1970; Fell, 1974). The duration of their free-swimming periods also varies greatly among species, from a few hours to twenty days in the laboratory. Thus in *H. panicea* and other species as well, it has not been possible to clearly elucidate the relationship between larval behavior and the habitats of the adult sponges. The sponge larvae may behave somewhat differently in their natural environments (Fell, 1974).

In the class Demospongiae, most sponges of the subclass Ceractinomorpha are viviparous. On the other hand, a larger part of other subclass Tetractinomorpha is oviparous (Levi, 1956, 1957; Bobojevic, 1966; Van de Vyver and Willenz, 1975; Reiswig, 1976; Watanabe, 1978). Marine sponges of the former subclass are common in the intertidal zone of temperate and tropical regions. In the intertidal zone habitat selection seems much more critical for free-swimming sponge larvae because the environmental conditions—light, seawater temperature, current, etc.—usually range more widely than on deeper bottoms (Meadows and Campbell, 1972). Embryos of viviparous sponges develop while being protected within the mesohyle for several weeks or months before they become swimming larvae. If eggs were released from an intertidal sponge, they might be carried far away from the intertidal region during the embryonic development thereby losing the chance to settle in a suitable habitat. Larvae released from intertidal sponges can swim actively, respond to environmental stimuli, and settle in a suitable habitat in a relatively short but critical free-swimming period. Therefore I suggest that viviparity is advantageous for the adaptation of sponges to intertidal existence.

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LITERATURE CITED

ALI, M. A. 1956. Development of the monoaxonid sponge, *Lissodendoryx similis* Thiele. *J. Madras Univ.* **B26:** 553–581.

AYLING, A. L. 1980. Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine demospongiae. *Biol. Bull.* **158:** 271–282.

BERGQUIST, P. R., M. E. SINCLAIR, AND J. J. HOGG. 1970. Adaptation to intertidal existence: reproductive cycles and larval behaviour in demospongiae. *Symp. Zool. Soc. Lond.* **25:** 247–271.

BOROJEVIC, R. 1966. Etude Expérimentale de la différentiation des cellules de l'éponge au cours de son développement. *Dev. Biol.* **14:** 130–153.

CHEN, W. T. 1976. Reproduction and speciation in *Halisarca.* Pp. 113–139 in *Aspects of Sponge Biology,* F. W. Harrigan and R. R. Cowden, eds. Academic Press, NY.

FELL, P. E. 1967. Sponges. Pp. 265–276 in *Methods in Developmental Biology,* F. H. Wilt and N. K. Wessels, eds. Thos. Y. Crowell Co., NY.

FELL, P. E. 1969. The involvement of nurse cells in oogenesis and embryonic development in the marine sponge, *Haliclona ecbasis. J. Morphol.* **127:** 133–150.

FELL, P. E. 1974. Porifera. Pp. 51–132 in *Reproduction of Marine Invertebrates,* Vol. 1, A. C. Giese and J. S. Pearse, eds. Academic Press, NY.

FELL, P. E., AND W. F. JACOB. 1979. Reproduction and development of *Halichondria* sp. in the Mystic estuary, Connecticut. *Biol. Bull.* **156:** 62–75.

HARRIGAN, J. F. 1972. Behaviour of the planula larva of the scleractinian coral *Pacillopora damicornis* L. *Am. Zool.* **12:** 723.

HYMAN, L. H. 1940. Metazoa of the cellular grade of construction—Phylum Porifera, the sponges. Pp. 284–364 in *The Invertebrates,* Vol. 1. McGraw-Hill, NY.

LEVI, C. 1951. L'oviparité chez les spongiaires. *C. R. Acad. Sci. Paris* **233:** 272–274.

LEVI, C. 1956. Étude des *Halisarca* de Roscoff. Embryologie et systématique dés demosponges. *Arch. Zool. Exp. Gen.* **93:** 1–181.

LEVI, C. 1957. Ontogeny and systematics in sponges. *Syst. Zool.* **6:** 174–183.

LEWIS, J. B. 1974. The settlement behaviour of planulae larvae of the hermatypic coral *Favia fragum* (Esper.). *J. Exp. Mar. Biol. Ecol.* **15:** 165–172.

MEADOWS, P. S., AND J. I. CAMPBELL. 1972. Habitat selection by aquatic invertebrates. *Adv. Mar. Biol.* **10:** 271–382.

MILLAR, R. H. 1971. The biology of ascidians. *Adv. Mar. Biol.* **9:** 1–100.

OLSON, R. R. 1983. Ascidian-*Prochloron* symbiosis: the role of larval photoadaptations in midday larval release and settlement. *Biol. Bull.* **165:** 221–240.

REISWIG, H. M. 1976. Natural gamete release and oviparity in Caribbean Demospongiae. Pp. 99–112 in *Aspects of Sponge Biology,* F. W. Harrison and R. R. Cowden, eds. Academic Press, NY.

SIMPSON, T. L. 1968. The biology of the marine sponge *Microciona prolifera* (Ellis and Solander). II. Temperature-related, annual changes in functional and reproductive elements with a description of larval metamorphosis. *J. Exp. Mar. Biol. Ecol.* **2:** 252–277.

VAN DE VYVER, G., AND P. WILLENZ. 1975. An experimental study of the life-cycle of the fresh-water sponge *Ephydatia fluviatilis* in its natural surroundings. *Wilhelm Roux' Arch. Dev. Biol.* **172:** 4–52.

WARBURTON, F. E. 1966. The behaviour of sponge larvae. *Ecology* **47:** 672–674.

WATANABE, H., AND C. C. LAMBERT. 1973. Larva release in response to light by the compound ascidians *Distaplia occidentalis* and *Metandrocarpa taylori. Biol. Bull.* **144:** 556–566.

WATANABE, Y. 1978. The development of two species of *Tetilla* (demospongiae). *Nat. Sci. Rep. Ochanomizu Univ.* **29:** 71–106.

WILSON, H. V. 1894. Observations on the gemmule and egg development of marine sponges. *J. Morphol.* **9:** 277–406.